THE EXPERIMENTAL TRANSMISSION OF CUTANEOUS LEISHMANIASIS TO MAN FROM *PHLEBOTOMUS PAPATASII*

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Sandflies have been suspected by various authors of being the transmitting agent of oriental sore. The first definite evidence in favour of the *Phlebotomus* theory of cutaneous Leishmaniasis was provided by Wenyon (1912) who found about six per cent. of the sandflies he dissected in Aleppo infected with *Herpetomonas* resembling cultural forms of Leishmania. Wenyon states that all intermediate stages of development between the small non-flagellated bodies and the fully developed flagellates occurred. Later Mackie (1914) found ten per cent. of *Phlebotomus minutus* in Assam infected with *Herpetomonas* but Patton (1922) has pointed out that oriental sore is not endemic in Assam. In Mesopotamia where oriental sore is common and sandflies are a pest, Patton (1919) states that *Herpetomonas* is present in *Phlebotomus papatasii* and *P. minutus*, and in 1922 the same author remarks on the presence of *Herpetomonas* in *Phlebotomus papatasii* and *P. minutus* in Palestine.

Additional evidence for the *Phlebotomus* theory was adduced by Acton (1919) who showed that the distribution of oriental sores on the body corresponds to the distribution of bites of *Phlebotomus*.

In Palestine the epidemological evidence for the *Phlebotomus* theory of transmission of oriental sores (Jericho Boils) is ambiguous but rather favourable to the *Phlebotomus* theory. Canaan (1916) who first demonstrated Leishman-Donovan bodies in oriental sores from Jericho considered that town to be the only endemic centre of cutaneous Leishmaniasis in Palestine. Later however Kligler (1923) reported three cases from Kantara, Dostrowsky (1925) described ten cases from Artuf and also found one case from Bethlehem and one from Mozza, a small village near Jerusalem.

The aetiology of the disease as described by Dostrowsky in Artuf is of great interest. The population of Artuf is one hundred and fourteen and until 1923 sandflies were not observed in Artuf according to the statement of Dr. E. Jaruslawsky, the local physician (sandflies when present in numbers never pass unobserved). In the summer of 1923 the insects had for the first time become a pest to the villagers. This fact raised Dr. Dostrowsky's suspicion that the *Phlebotomus* was the carrier of oriental sores.

In June, 1924, one of us (A.) on Dr. Dostrowsky's suggestion examined the village and found every house infested with *Phlebotomus*. Three species *P. papatasii*, *P. minutus* and *P. perniciosus* were present, *P. papatasii* being by far the commonest. Dr. Dostrowsky then examined the population (ninety-seven) of an Arab village only one hundred metres from Artuf. Material was taken from every suspicious papule and given to one of us (A.). On examination Leishman-Donovan bodies were absent in every case. An examination of this village made by one of us (A.) did not reveal a single specimen of *Phlebotomus*. This may be explained by the fact that this village consists of mud huts without windows and uniformly dark in the interior, while the first village consists of fairly modern houses with whitewashed interiors, the furniture and clothes producing shade and contrast to the whitewashed walls. In Jerusalem, Jericho and Artuf it was noted that *Phlebotomus* prefers the shade produced by contrast to uniform darkness.

In Jericho *Phlebotomus papatasii* and *P. minutus* occur in large numbers, the former species predominating. *P. perniciosus* is absent or very rare. In Mozza *P. papatasii* and *P. perniciosus* are both common. It would seem then that *P. papatasii* is the carrier of cutaneous Leishmaniasis in Palestine, it being the only species common to three localities where cutaneous Leishmaniasis occurs. Schroetter, (1923), incriminated an insect which he called *Phlebotomus el Ghor* as the carrier in Jericho but Martini has shown that this insect is not a *Phlebotomus* and is incapable of biting.

Against the *Phlebotomus* theory is the fact that cutaneous Leishmaniasis is absent from many localities in Palestine where sandflies are a plague, e.g., the village of Rehoboth containing twelve hundred inhabitants where all three Palestinian species

of *Phlebotomus* abound. In Haifa (population about 30,000) which is the place most infested with sandflies in the whole of Palestine, all three Palestinian species being present, oriental sore is hitherto unknown. Still more striking is the fact that up to the present no locally acquired cases of cutaneous Leishmaniasis have been noted in Jerusalem itself. There are always a number of cases of cutaneous Leishmaniasis in Jerusalem from Jericho, Bagdad, Aleppo and Persia. In addition *Phlebotomus papatasii* is very common, *P. perniciosus* also occurs, and *P. minutus* is very rare, i.e., there are, according to the *Phlebotomus* theory, ideal conditions for the spread of the disease. Were even a small number of cases present they would not pass unnoticed for all classes of the population of Jerusalem assiduously attend the numerous clinics of the city for even the most trivial maladies and physicians are on the look-out for a case of locally acquired oriental sore.

It would seem then that on the assumption that a *Phlebotomus* sp. is the carrier of the disease in nature a third and hitherto unknown factor apart from human cases and insect carriers is necessary for the spread of the disease. What this factor is still remains to be investigated.

EXPERIMENTAL TRANSMISSION TO HUMAN BEINGS

Sergent, Parrot, Donatien and Béguet (1921) first described the experimental transmission of oriental sore to a human being. These authors divided five hundred and fifty-nine sandflies into twenty-three batches, crushed them in saline and used the resulting material for inoculation into the arms of twenty-three volunteers. The material was collected at Biskra, an endemic centre of oriental sore, and the experiments were performed at Algiers where oriental sore is unknown.

Only one experiment from a batch of seven specimens of *Phlebotomus papatasii* gave a positive result. The experiment was performed on the 20th August, 1921; two months and twenty days later a papule was noted, and on the following day numerous Leishman-Donovan bodies were found in the papule.

In October, 1924, one of us (A.) commenced an examination of *Phlebotomus* in Jericho. Two hundred and twenty specimens of

Phlebotomus from Jericho, of which one hundred and seventy-four were females, were dissected during October to December, 1924, and three females gorged with mammalian blood were found to contain *Herpetomonas* in their midgut. All stages from non-flagellated forms to long flagellated forms were noted.

The following is a method recommended for the examination of *Phlebotomus* for *Herpetomonas*. If the insect contains no blood, cut off the terminal abdominal segment, stroke the upper surface of the abdomen with a needle to push out the ova, gently pull the head with one needle, holding the other needle against the upper surface of the thorax. In this way the head, oesophagus, salivary glands, oesophageal diverticulum and midgut are removed together, and the individual parts of the alimentary tract can be examined for *Herpetomonas*. If the midgut contains blood gently pull the head away from the thorax. The salivary glands, oesophagus, and oesophageal diverticulum and occasionally the upper part of the midgut will come away together with the head. The rest of the alimentary canal can then be pulled out from the hind end in the usual way.

In December, 1924, sandflies became very rare in Jericho and on the 20th December, 1924, a search through the whole town by a trained assistant yielded only four specimens of *P. papatasii*. Subsequent monthly examinations revealed no sandflies until April 20th, 1925. They were not found on April 4th, 1925, but on April 20th, 1925, they were numerous, *Phlebotomus papatasii* only being present. *Phlebotomus minutus* appeared towards the end of June.

A batch of one hundred and ninety-eight sandflies of which one hundred and ninety-one were *P. papatasii* (one hundred and seventy-five females and sixteen males) and seven *P. minutus* (six females and one male) were collected in Jericho on the 25th June, 1925, and brought to Jerusalem for dissection. Of this batch only one specimen, a female *P. papatasii*, was found to contain *Herpetomonas*. The insect contained no trace of blood and the abdominal cavity was full of ripe or almost ripe eggs. The whole alimentary tract was found to be swarming with *Herpetomonas*. Flagellates were found in the oesophagus, oesophageal diverticulum, midgut and hindgut. They were especially numerous in the upper part of the midgut where swarms of parasites appeared to be attached to the posterior surface of the oesophageal valve, so much so that some parasites appeared at first sight on examination in the fresh preparation to be intracellular. (The oesophageal valve is a well-marked structure in *Phlebotomus*.)

The fact that flagellates were noted in the oesphageal diverticulum is of great interest for in freshly dissected specimens it is frequently seen that waves of peristalsis pass from the posterior end of the oesophageal diverticulum towards the oesophagus, which in *Phlebotomus* is very short, so that the oesophageal diverticulum practically opens into the pharynx. It is thus possible that flagellates may be propelled into the pharynx and buccal cavity and the possibility of a direct infection by the bite of a sandfly must be considered. Hitherto it has been generally held that infection takes place only through the crushing of an infective *Phlebotomus* on the skin, a theory which is very feasible in view of the large number of sandflies crushed on the skin (e.g. Cornwall, 1922). Another point of great interest was observed, i.e. the flagellates were not polymorphic as in the cases observed by Wenyon (1912) and by one of us (A.) in 1924, but they were all elongated and had long flagella. The oesophagus, together with the piece of oesophageal diverticulum and the upper part of the midgut, containing the oesophageal valve, were dissected away together, and another part of the midgut behind the oesophageal valve was dissected off separately; these parts were placed on separate slides, fixed in absolute alcohol, stained overnight with Giemsa, differentiated with a 0·02 per cent. solution of acetic acid and permanently mounted.

The remainder of the material was used for inoculation into the forearm of a volunteer. The volunteer had previously been exposed for three years (1917-1920) to oriental sore in Mesopotamia without contracting the disease. Two points on the skin of the left forearm were scarified and material containing flagellates was rubbed into each on the 26th of June, 1925. On the 31st July, 1925, a small papule which would normally have passed unobserved was noted on one of the inoculated points and on examination Leishman-Donovan bodies were found. The incubation period was thus less than half that noted by the Sergents and their collaborators (1921).

The dissection of individual sandflies and experimenting with material from one infected individual is more satisfactory than crushing large numbers in saline and experimenting with the product, for in the latter case it is impossible to know whether a negative result is due to the fact that none of the sandflies contained

Herpetomonas or whether the *Herpetomonas* was non-infective. From April to June, 1925, a thousand and thirty-seven sandflies collected in Jericho were dissected and found negative for *Herpetomonas ;* on the 9th of June another two hundred sandflies from Jericho were collected and on dissection found negative. Thus the experiment of crushing more than twice the number of sandflies from an endemic centre used by the Sergents and their collaborators and inoculating a single volunteer would have given a negative result.

Nothing was noted on the other site of inoculation; nevertheless the part was scraped and on the 31st of July, 1925, examined for Leishman-Donovan bodies with a negative result. It was again examined on the 4th of August, 1925, with a negative result.

The successful results of the above experiment and the experiment of the Sergents and Parrot, Donatien and Béguet prove that human beings can be infected with oriental sore by inoculation with *Herpetomonas* from *Phlebotomus papatasii,* but the epidemiological evidence that *P. papatasii* is the only carrier of the disease in nature is not yet complete.

In view of the successful infection with Leishmaniform parasites by injection of *Herpetomonas ctenocephali* into mice, rats, and a dog, and by injection of *Crithidia fasciculata* into mice and rats (Laveran and Franchini, 1913), by injection of *Herpetomonas jaculum* from the water scorpion *Nepa cinerea* into mice, by feeding a puppy on dog fleas (Fantham and Porter, 1915), by injecting *Herpetomonas jaculum* and *H. culicis* into birds (Fantham and Porter, 1915) the possibility of other sources of infection apart from *P. papatasii* must be considered in spite of the fact that the distribution of sandflies corresponds more closely than that of any other biting insect to the distribution of oriental sore. It must be pointed out, however, that among others Hoare (1921) working with *Crithidia melophagia, Herpetomonas jaculum* and *H. calliphorae* on mice, sticklebacks, newts and frogs, and Shortt (1923) working with *Herpetomonas ctenocephali* and *H. lucilae,* failed to infect monkeys, dogs, rabbits, rats, mice, pigeons and frogs.

The question of other insect carriers of oriental sore apart from *Phlebotomus papatasii* can only be cleared up in the future by direct experiments on human skin with flagellates from various insects.

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